

## SEQUENCE LISTING

<110> Brookhaven Science Associates  
Shanklin, John

<120> Mutant Fatty Acid Desaturase and Methods for Directed Mutagenesis

<130> CIP of 09/328,550 filed June 9, 1999; which was a CIP of 09/233,856  
filed January 19, 1999

<150> 09/328,550

<151> 1999-06-09

<160> 13

<170> PatentIn version 3.1

<210> 1

<211> 363

<212> PRT

<213> Ricinus communis

<220>

<221> misc\_feature

<223> ricinus communis delta 9 18:0 Acyl ACP Desaturase

<400> 1

Ala	Ser	Thr	Leu	Lys	Ser	Gly	Ser	Lys	Glu	Val	Glu	Asn	Leu	Lys	Lys
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Pro	Phe	Met	Pro	Pro	Arg	Glu	Val	His	Val	Gln	Val	Thr	His	Ser	Met
			20					25					30		

Pro	Pro	Gln	Lys	Ile	Glu	Ile	Phe	Lys	Ser	Leu	Asp	Asn	Trp	Ala	Glu
		35					40					45			

Glu	Asn	Ile	Leu	Val	His	Leu	Lys	Pro	Val	Glu	Lys	Cys	Trp	Gln	Pro
	50					55					60				

Gln	Asp	Phe	Leu	Pro	Asp	Pro	Ala	Ser	Asp	Gly	Phe	Asp	Glu	Gln	Val
65				70						75				80	

Arg	Glu	Leu	Arg	Glu	Arg	Ala	Lys	Glu	Ile	Pro	Asp	Asp	Tyr	Phe	Val
			85					90						95	

Val	Leu	Val	Gly	Asp	Met	Ile	Thr	Glu	Glu	Ala	Leu	Pro	Thr	Tyr	Gln
			100					105					110		

Thr Met Leu Asn Thr Leu Asp Gly Val Arg Asp Glu Thr Gly Ala Ser  
115 120 125

Pro Thr Ser Trp Ala Ile Trp Thr Arg Ala Trp Thr Ala Glu Glu Asn  
130 135 140

Arg His Gly Asp Leu Leu Asn Lys Tyr Leu Tyr Leu Ser Gly Arg Val  
145 150 155 160

Asp Met Arg Gln Ile Glu Lys Thr Ile Gln Tyr Leu Ile Gly Ser Gly  
165 170 175

Met Asp Pro Arg Thr Glu Asn Ser Pro Tyr Leu Gly Phe Ile Tyr Thr  
180 185 190

Ser Phe Gln Glu Arg Ala Thr Phe Ile Ser His Gly Asn Thr Ala Arg  
195 200 205

Gln Ala Lys Glu His Gly Asp Ile Lys Leu Ala Gln Ile Cys Gly Thr  
210 215 220

Ile Ala Ala Asp Glu Lys Arg His Glu Thr Ala Tyr Thr Lys Ile Val  
225 230 235 240

Glu Lys Leu Phe Glu Ile Asp Pro Asp Gly Thr Val Leu Ala Phe Ala  
245 250 255

Asp Met Met Arg Lys Lys Ile Ser Met Pro Ala His Leu Met Tyr Asp  
260 265 270

Gly Arg Asp Asp Asn Leu Phe Asp His Phe Ser Ala Val Ala Gln Arg  
275 280 285

Leu Gly Val Tyr Thr Ala Lys Asp Tyr Ala Asp Ile Leu Glu Phe Leu  
290 295 300

Val Gly Arg Trp Lys Val Asp Lys Leu Thr Gly Leu Ser Ala Glu Gly  
305 310 315 320

Gln Lys Ala Gln Asp Tyr Val Cys Arg Leu Pro Pro Arg Ile Arg Arg  
325 330 335

Leu Glu Glu Arg Ala Gln Gly Arg Ala Lys Glu Ala Pro Thr Met Pro

340

345

350

Phe Ser Trp Ile Phe Asp Arg Gln Val Lys Leu  
 355 360

<210> 2  
 <211> 1092  
 <212> DNA  
 <213> Ricinus communis

<220>  
 <221> misc\_feature  
 <223> residues 138 to 1239 of open reading frame

<400> 2  
 gcctctaccc tcaagtctgg ttctaaggaa gttgagaatc tcaagaagcc tttcatgcct 60  
 cctcgggagg tacatgttca ggttacccat tctatgccac cccaaaagat tgagatcttt 120  
 aaatccctag acaattgggc tgaggagAAC attctgggtc atctgaagcc agttgagaaa 180  
 tgttggcaac cgcaggattt tttgccagat cccgcctctg atggatttga tgagcaagtc 240  
 agggaactca gggagagagc aaaggagatt cctgatgatt attttgttgt tttggttgga 300  
 gacatgataa cgaagaagc ccttcccact tatcaaaca tgctgaatac cttggatgga 360  
 gttcgggatg aaacaggtgc aagtcctact tcttgggcaa tttggacaag ggcatggact. 420  
 gcggaagaga atagacatgg tgacctctc aataagtatc tctacctatc tggacgagtg 480  
 gacatgaggc aaattgagaa gacaattcaa tatttgattg gttcaggaat ggatccacgg 540  
 acagaaaaca gtccatacct tgggttcac tatacatcat tccaggaaag ggcaaccttc 600  
 atttctcatg ggaacactgc ccgacaagcc aaagagcatg gagacataaa gttgggtcaa 660  
 atatgtggta caattgctgc agatgagaag cgccatgaga cagcctacac aaagatagtg 720  
 gaaaaactct ttgagattga tctgatgga actgttttgg cttttgctga tatgatgaga 780  
 aagaaaattt ctatgcctgc acacttgatg tatgatggcc gagatgataa tctttttgac 840  
 cacttttcag ctgttgcgca gcgtcttgga gtctacacag caaaggatta tgcagatata 900  
 ttggagttct tgggtggcag atggaagggt gataaactaa cgggcctttc agctgaggga 960  
 caaaaggctc aggactatgt ttgtcggtta cctccaagaa ttagaaggct ggaagagaga 1020  
 gctcaaggaa gggcaaagga agcaccacc atgcctttca gctggatttt cgataggcaa 1080  
 gtgaagctgt ag 1092

<210> 3  
 <211> 34  
 <212> DNA  
 <213> Artificial

<220>  
 <221> misc\_feature  
 <223> PCR primer; sequence flanking unique XbaI site at the 5' end of the open reading frame

<400> 3  
 gtgagcggat aacaatttca cacagtctag aaat 34

<210> 4  
 <211> 72  
 <212> DNA  
 <213> Artificial

<220>  
 <221> misc\_feature  
 <222> (56)..(57)  
 <223> PCR primer is a degenerate oligonucleotide in which "n" indicates the presence of either C, A, T or G at that nucleotide position

<400> 4  
 ccaaattgcc caagacgtcg gacttgcacc tgtttcatcc cgaactccat ccaamnatt 60  
 cagcattggt tg 72

<210> 5  
 <211> 31  
 <212> DNA  
 <213> Artificial

<220>  
 <221> misc\_feature  
 <223> PCR primer

<400> 5  
 gaaacaggtg caagtccgac gtcttgggca a 31

<210> 6  
 <211> 26  
 <212> DNA  
 <213> Artificial

<220>  
 <221> misc\_feature  
 <223> PCR primer

<400> 6  
gttttctgtc cgcggatcca ttcttg

26

<210> 7  
<211> 34  
<212> DNA  
<213> Artificial

<220>  
<221> misc\_feature  
<223> PCR primer

<400> 7  
gtgagcggat aacaatttca cacagtctag aaat

34

<210> 8  
<211> 30  
<212> DNA  
<213> Artificial

<220>  
<221> misc\_feature  
<223> PCR primer

<400> 8  
cacgaggccc ttctgtcttc aagaattctc

30

<210> 9  
<211> 28  
<212> DNA  
<213> Artificial

<220>  
<221> misc\_feature  
<223> PCR primer

<400> 9  
ttgataagtg ggaagggtt cttccgtt

28

<210> 10  
<211> 66  
<212> DNA  
<213> Artificial

<220>  
<221> misc\_feature  
<222> (41)..(43)  
<223> PCR primer is a degenerate oligonucleotide in which "n" indicates the presence of either C, A, T or G and in which "k" indicates the presence of either T or G.

<220>  
 <221> misc\_feature  
 <222> (32)..(34)  
 <223> PCR primer is degenerate oligonucleotide in which "n" indicates the presence of either C, A, T, or G at that nucleotide position and in which "k" indicates either T or G

<220>  
 <221> misc\_feature  
 <222> (44)..(46)  
 <223> PCR primer is a degenerate oligonucleotide in which "n" indicates the presence of either C, A, T, or G at that nucleotide position and in which "k" indicates the presence of either T or G.

<400> 10  
 aacggaagaa gcccttccca cttatcaaac annkctgaat nnknnkgatg gagttcgga 60  
 tgaaac 66

<210> 11  
 <211> 26  
 <212> DNA  
 <213> Artificial

<220>  
 <221> misc\_feature  
 <223> PCR primer

<400> 11  
 tccattcctg aaccaatcaa atattg 26

<210> 12  
 <211> 70  
 <212> DNA  
 <213> Artificial

<220>  
 <221> misc\_feature  
 <222> (22)..(24)  
 <223> PCR primer in a degenerate oligonucleotide in which "n" indicates the presence of either C, A, T or G at that nucleotide position and in which "k" indicates the presence of either T or G at that nucleotide position.

<220>  
 <221> misc\_feature  
 <222> (28)..(30)  
 <223> PCR primer in a degenerate oligonucleotide in which "n" indicates the presence of either C, A, T or G at that nucleotide position

and in which "k" indicates the presence of either T or G at that nucleotide position.

<220>  
 <221> misc\_feature  
 <222> (49)..(51)  
 <223> PCR primer in a degenerate oligonucleotide in which "n" indicates the presence of either C, A, T or G at that nucleotide position and in which "k" indicates the presence of either T or G at that nucleotide position.

<400> 12  
 ttgattgggt caggaatgga tnnkcggnnk gaaaacagtc catacctttn kttcatctat 60  
 acatcattcc 70

<210> 13  
 <211> 30  
 <212> DNA  
 <213> Artificial

<220>  
 <221> misc\_feature  
 <223> PCR primer

<400> 13  
 gcaaaagcca aaacggtacc atcaggatca 30